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Heritable Health: An Exploration of Parental Epigenetics and their Impact on Individual and Public Health

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For many of us, our parents were there throughout our childhood to feed, clothe, nurture, encourage, and guide us. But before we were even born, our parents provided us with information that played a major role in determining who we became: our genome. Each of us received half of our genome from each parent and these two sets of genetic information came together to determine how our body looks and functions. Our inherited genome made it possible for characteristics, such as eye color, hair color and height, to pass from parent to offspring. None of this information should come as a real surprise to anyone, but some people might be surprised to learn there is another way for us to inherit biological information from our parents besides DNA.

Epigenetics, a relatively new field of biology that emerged in the mid-20th century, demonstrates that are parents are passing on more to us than just DNA. A large body of ever expanding epigenetic research is creating a powerful image of just how much of our parents' experiences are passed onto us through epigenetic processes. When these experiences are passed down from parent to offspring, their transmission is often marked by some "epigenetic effect." Epigenetic research demonstrates the existence both maternal and paternal epigenetic effects and together, these two fields create the body of research known as parental epigenetic effects. For both maternal and paternal research, there is a wide variety of literature demonstrating that the health status of parents affects the health status of their offspring. These epigenetic effects are as varied as the factors and influences that cause them.

However, the great level of diversity in parental epigenetic effects causes some problems in the field. Because of the breadth of research, it is hard to find sources that bring all of the research and demonstrated effects together in one place. While it is not

realistic to compile *all* the available research, we need a source that begins to piece together the wide variety research on parental epigenetics effects. Another issue that faces parental epigenetic research is that the body of research is new enough that it is hard to know what it will for the big picture. The more we understand how these effects are transmitted and the stronger the evidence becomes for the connections between parental experience and health problems in offspring, the more likely it is that epigenetic research will expand to influence disciplines beyond itself.

The goal of this thesis is to compile a sample of the available research on parental epigenetic effects and ultimately analyze the potential for this research to impact public health and health policy. The first step to achieve this goal is to explore the history and mechanism of epigenetics and provide enough information for a reader to comfortably approach the selection of research included in this document. The research I include in this thesis focuses on parental epigenetic effects that are created by relatively common health problems and environmental factors, such as smoking and nutrition, and how these lead to negative health impacts in offspring. Because all these studies relate to the impact of paternal and maternal epigenetic effects on the health of their offspring, a logical expansion of this research is to examine the impact this research could have on public health and policy. Therefore, the last section of this thesis addresses some possible implications this research has on various aspects of the field of public health.

Epigenetics: A brief history and background

The field of epigenetics is a relatively new and quickly advancing field within biology. At the beginning of the 20th century, developmental biology and genetics were two completely separate fields and it was not appreciated how interconnected they might be. However, it became increasingly evident to some scientists that there was in fact overlap between the fields and they could possibly come together in a common discipline. Conrad Waddington, a professor at Edinburgh University, was experienced and knowledgeable in both fields, and this provided him with insight that made him instrumental in the creation of a field that unified genetics and embryology.

In his 1942 paper, *The canalization of development and the inheritance of acquired characters,* Conrad Waddington set in motion creation of the unified field of modern epigenetics (Gilbert and Epel 2009). It was in this article that he coined the term epigenesis from the Greek words "epi" and "genesis" which roughly translates to, "being above generation, birth, or origin." Waddington later modified the term epigenesis to epigenetics and altered the meaning to mean, "above the genetic sequence." In this same paper, Waddington coined the term epigenotype, a term he used to describe the total developmental system consisting of interrelated developmental pathways through which the adult form of an organism is realized (Waddington 1942). Waddington believed that epigenetics, which incorporated the concepts of epigenotype, would become "the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being (Dupont 2009)."

Waddington's idea that genes were fundamental regulators of development was paradigm changing within the field of biology. As more scientists expanded research within

the new field of epigenetics, most agreed that in general, observations that were not easily explained in genetic terms but had a heritable component fell into the realm of epigenetics. However, there wasn't an agreed upon definition of epigenetics among them. While Waddington was the first to coin the term and give us a definition, not everyone agreed upon this definition. Through increased technology and advancements in the both genetics and developmental biology, scientists have generated better definitions for what epigenetics really is. Today, epigenetics is typically defined as "the study of heritable changes in gene expression that are not due to changes in the DNA" (Gilbert and Epel 2009). Heritable changes can be viewed at a micro level of being transferred through daughter cells, which are cells that are the product of cell replication that have receive identical copies of the parent (original) cell's DNA, and at a more macro level of being transferred to future offspring of an organism, sometimes for multiple generations.

Before getting any further into what epigenetic is, it is important to have a basic understanding of our genome. Every living organism on the planet contain a set of biological instructions, and these instructions are housed in deoxyribonucleic acid, or DNA and a complete set of DNA makes up a genome. DNA is a two-stranded structure, known as the double helix, and is made up of four different types of nucleotides. The four bases, guanine (G), cytosine (C), adenine (A) and thymine (T), and the order that they occur in within the DNA sequence are what determine what the DNA codes for. Within the DNA sequence, there are regions of the sequence that codes for the creation of a protein known region as a gene. Through a process called gene expression, these sequences are read and the information is used to create a protein ("Deoxyribonucleic Acid (DNA) Fact Sheet"). Within each cell, only a small number of genes in the entire genome are actually turned on.

Gene expression is the term used to describe the variations in what genes are active in a particular cells genome. The actual turning of genes on and off in different patterns throughout the genome is a process known as gene regulation. The gene regulation is critical in cell differentiation because gene regulation occurs during development of an organism and is what determines how cells both look and function ("Can Genes Be Turned on and off in Cells?").

Epigenetics is an incredibly important part of our development because epigenetic mechanisms play an instrumental role in cell differentiation because epigenetic changes can lead to changes in gene expression. A multicellular organism is made up of an extremely vast and diverse collection of cells, all of which descended from the same single cell. Somehow, the genome of the original single cell that is maintained through all future descendants is able to create the extreme diversity of cellular. Whatever changes are occurring to the genome to create the many cell types leads are stable changes that are maintained in future cell generations. An example of how these changes are maintained is our skin. Skin cells are constantly dying off and being replaced by new cells that are already programmed to be skin cells because they received that information from the generation before them. The changes that occurred in the parent cell's genotype to make it a skin cell are remembered through mitosis and these "memories" ensure that all daughter cells also become skin cells. Epigenetic modifications help makes this differentiation and memory of change possible. The epigenetic modifications are stable enough to remain on the genetic code for decades and can even be passed through the germline.

Conrad Waddington understood that cell differentiation as one of the developmental processes mediated by altered gene expression. To explain the process, Conrad

Waddington created his “epigenetic landscape” (Waddington 1957). This epigenetic landscape provides a visual for the metaphor he used to explain what happens to a cell as it becomes differentiated.

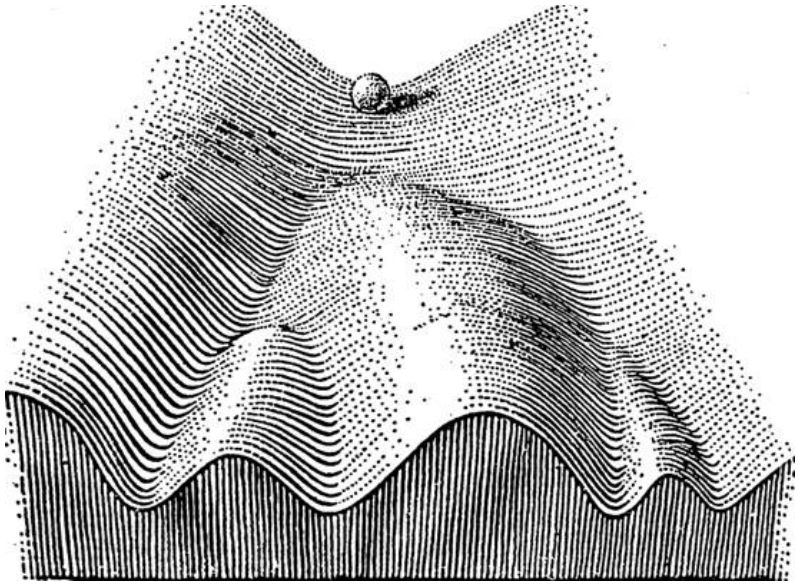

Figure 1. The visual representation of the epigenetic landscape by Conrad Waddington. The image shows a ball at the top of an incline and below has several different paths that it can take, a process that he compared to cell differentiation. (Waddington 1957).

In his epigenetic landscape, Waddington compared the process of cell differentiation to a ball rolling down a hillside (Figure 1). In this process, there is a ball at rest at the top of a hillside. When the ball begins to roll, it would have several different troughs that it could roll down. Each one of these troughs represents the different cell fates open to an undifferentiated cell. As the ball rolls down the hill, it must pick a pathway to roll down the hill. Along each pathway, there are further divisions in the trough and the ball must continue to pick only one to travel down. With each choice, there are fewer and fewer options remaining and the troughs that were once available to it are left behind. The ball continues to get farther and father away from the original starting point and it would be

nearly impossible for the ball to reach the top again once it has reached the bottom of the hillside.

With each change a cell undergoes during differentiation, it has fewer paths available to it. In the beginning, a stem cell has dozens of paths it could take, but once it chooses any of those paths, it must continue on with that path and has lost the ability to access other cell fates. Even though the cell has lost access to these other fates, it still has the same genome as it started with. As the cell becomes more and more differentiated and father from the original zygote, it becomes increasingly harder for the cell to return to its original form. Because of this, the new, fully differentiated cell will maintain its phenotype for the remainder of its lifetime. Also, all future generations of this cell will start life out expressing the same phenotype as the parent cell because it would be almost impossible for the cell to discard all the changes to the genome they inherit from the parent cells that led to the cell differentiation in the first place.

Epigenetic mechanisms such as DNA methylation and histone modifications, mechanisms that I will explain in more detail shortly, act on a cell's genome the throughout the process of cell differentiation. These mechanisms have the ability to and turn certain genes on and off, which leads them to their differentiated state. While the cell has maintained the same overall genome, the genes are now being expressed differently because of epigenetic changes. These changes are stable, and it is hard to reverse the effects of the epigenetic changes, like how it would be hard to get the ball back to the top of the hill. However, studies have proven that it is not impossible take a fully differentiated cell and undifferentiated it.

During the 1950s, a series of experiments demonstrated that contrary to previous belief, a differentiated cell could be returned to its undifferentiated form. Popular thought at the time within the field of developmental biology was that when as a cell progressed down one pathway of differentiation, genes that were no longer being expressed were lost from the genome (Gurdon 2003). In a 1952, Briggs and King performed the first experiment that allowed researchers start to answer the question of whether or not genetic material in a cell is lost during development and cell differentiation. In their experiment, they designed a technique to transplant a nucleus from one cell into an 'empty' cell without damaging either the donor cell nucleus or the recipient cell cytoplasm (Briggs and King 1952).

The results of this study showed that when transferring a nucleus from a cell at the very beginning of its development, known as a blastula cell, to a cell without a nucleus, they could create viable embryos in about 40% of the transfers they performed. However, when they repeated the process using cells from a later stage in development, known as gastrula cells, they did not see anywhere near the same success rates, at only 6% of the embryos being viable. From this 6% of viable embryos, all the resulting tadpoles experienced some level of abnormal development (Briggs and King 1952). These results suggest that even though gastrula cells are in a relatively early stage of development, changes have occurred to the nuclei of these cells that makes them unviable substitutes for the nuclei of a zygote cell. This experiment did not demonstrate whether or not the changes to the nuclei were permanent of not, but did provide future researchers with a technique to test this hypothesis.

Just a few years after Briggs and King demonstrated their nuclei transfer technique, John Gurdon began to performing experiments that further demonstrated the ability to transfer nuclei between cells and create viable embryos and experienced success in many of his studies (Gurdon et al. 1958, Gurdon 1961). However, it was in his 1962 study that Gurdon changed our understanding of cell differentiation. In this experiment, Gurdon used a process called somatic cell nuclear transfer (SCNT) to removed the nucleus from the adult intestinal epithelial cell and inserted it into an unfertilized egg that's had its own nucleus removed. If tadpoles developed from the egg with the replaced nucleus, this would mean that it is possible for a nucleus from a fully differentiated cell could act as a substitute for a nucleus in a zygote. If this were true, these results would discredit the popular idea at the time that genetic information is lost as the cell differentiates. However, if no growth resulted from the cells with the donor nuclei, these results would not have necessarily supported the idea genetic information is lost. The process of SCNT was incredibly challenging with the technology of the time and there was a chance that in the process of transferring the nucleus from one cell to the other would kill the nucleus. There would be no way to know whether or not the lack of tadpole development was from poor lab technique or because the genetic material was irreversibly lost (Gurdon 1962).

In this experiment, John Gurdon did manage to produce viable toads by transferring the nucleus of an intestinal epithelial cell into an undifferentiated cell. However, the cleavage and embryonic development of these embryos were much more abnormal than the embryos that resulted from the control group blastula transfers. However, serial transplantation of the nuclei that had originally resulted in abnormal development of embryos created normally developed tadpoles after serial transfer. Between the initial

transfers and serial transfers, 7% of these transfers resulted in the formation of normally developed tadpoles. That means that 7% of the total transfers contained all the genetic information necessary to create a normal and fully functioning tadpole (Gurdon 1962).

While not an overwhelmingly high rate of the transfers resulted in tadpoles, these results suggest that a differentiated cell has the same amount of genetic information as the initial zygote. This means that during the process of differentiation, the genes that are turned off in the cell are maintained in the genome and not lost or permanently inactivated. By comparing the success of the transfers using the intestinal epithelial cells to a control group of transfers performed using nuclei taken from blastula cells, we see that while it is more difficult to create a whole animal from a more specialized cell, Gurdon proved it is not impossible. Returning to the idea of the epigenetic landscape, Gurdon proved that while it's hard to get the ball back up to the top, if you can get it there, the ball didn't experience permanent changes from going down the hillside and could take the journey again.

When Gurdon and his contemporaries were performing these experiments, the term epigenetics was very new and poorly defined and no one knew what mechanism made cells specialized. However, with the improvements the field of genetics, we have learned how these phenotypes are altered without altering the genotype. The two main mechanisms in epigenetics are DNA methylation and histone modification.

As the name suggests, DNA methylation is the process in which a methyl group is added to another chemical, and in relation to epigenetics, this chemical is DNA. The addition of a methyl group, which is a carbon atom with three hydrogen atoms linked to it (CH_3), is a very small modification to the DNA. Methyl groups can only attached to the 5' carbon of the DNA base cytosine in regions know as CpG islands (Figure 2).

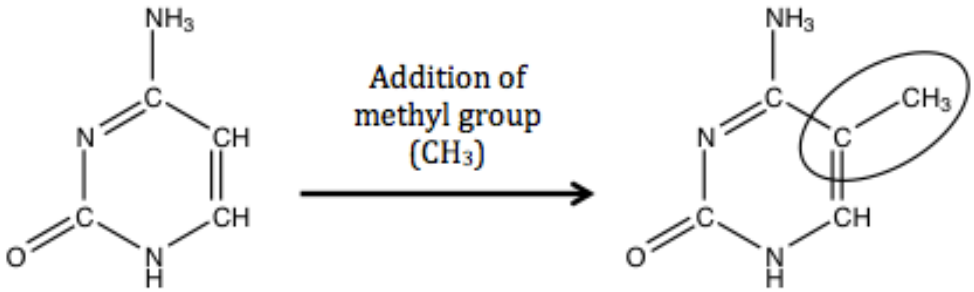

Figure 2. Displays the location a methyl group is added onto the DNA base Cytosine.

A CpG island is an area with high frequency of CG sites, which means that the cytosine and guanine are right next to one another, which is necessary for methylation to occur. Cytosine is unique because it is the only DNA base that can be methylated. Three different enzymes make the chemical reaction of attaching the methyl group to the cytosine possible: DNMT1, DNMT3A and DNMT3B. The DNMT stands for DNA methyltransferase and are considered examples of epigenetic writers because they help create the epigenetic code through methylation.

It is important to remember that no part of methylation changes the genetic code; it just adds a chemical group onto the nucleotide. While the addition of the methyl group doesn't change the code itself, it does have a huge impact, even though it is such a small modification. The effect of methylation is to inhibit the expression of the gene to which it is attached (Francis 2011). Early experiments on methylation showed that injecting DNA into a nucleus of a cell, the DNA that was more methylated resulted in less RNA being transcribed. This showed that high levels of methylation are correlated with certain genes being switched off. Later experiments showed that when a gene had been switched off, or

silenced, the promoter regions of these genes contained significantly larger amounts of methylated cytosines in comparison to actively transcribed genes (Carey 2012), which supports the idea that the more methylated a gene is, the less active it is. Variations in the patterns of methylation are responsible for the variation of gene silencing among cells.

Methylation patterns are heritable and are restored each time the DNA is replicated. The enzyme DMNT1 is a critical in the maintaining the methylation patterns. After the DNA is separated and replicated, it is then checked by DMNT1. When the DMNT1 checks the DNA, the enzyme is able to detect when the methylation pattern on one strand of DNA is not matched on the newly synthesized strand. DMNT1 then adds the missing methylation on the other strand so the pattern is continued. This means that the daughter cells have acquired the same methylation pattern as the parent cell, so the same genes will be suppressed over multiple generations.

Genomic imprinting is another way in which methylation affects development. For some genes, only one copy of the gene will be expressed, and the expression of what gene is expressed is dependent on which parent the gene came from. Genomic imprinting is the process in which the active gene is transmitted from one parents over the other parent. Imprinting occurs by a pattern of methylation where the copy of the gene that is inactivated is covered with methyl groups. The process of imprinting is limited because it does not occur on every chromosome. The process of genetic imprinting takes place before fertilization within the egg and the sperm. This means that in an offspring, if they receive a chromosome that was imprinted, or inactivated, from the sperm, the copy of the chromosome from the egg will be active. This paternal pattern of imprinting will remain after fertilization and throughout development and all cells in the body will reflect this

imprinting. However, when a gamete is formed, the genes in the gamete must reflect their gender. That means the genes in the egg must be maternally imprinted where the genes in the sperm must be paternally imprinted. This requires a conversion to occur in imprints so the genes reflect their gender, not their parents ("Genomic Imprinting"). This switch demonstrates that gene imprinting through methylation is actually a reversible form of gene inactivation.

The other major mechanism of epigenetics is histone modification. Histones are globular proteins, which means they are proteins that are folded into ball-like shapes. Each one of these ball-like structures has an attached chain of amino acids called a histone tail. The four histones, H2A, H2B, H3 and H4, are of particular interests in regards to epigenetics right now. These four histones form a histone octamer, where two of each of the four globular histones come together and form a cube-like structure with two layers, with four histones in each layer. Histones were thought of as "packaging" proteins. The length of DNA found in the cell is so great that to be contained in the cell, it is wrapped around the histones for storage. When the DNA is coiled tightly around the histone octamer, this forms what is called a nucleosome, as shown in Figure 3 (Tammen 2012).

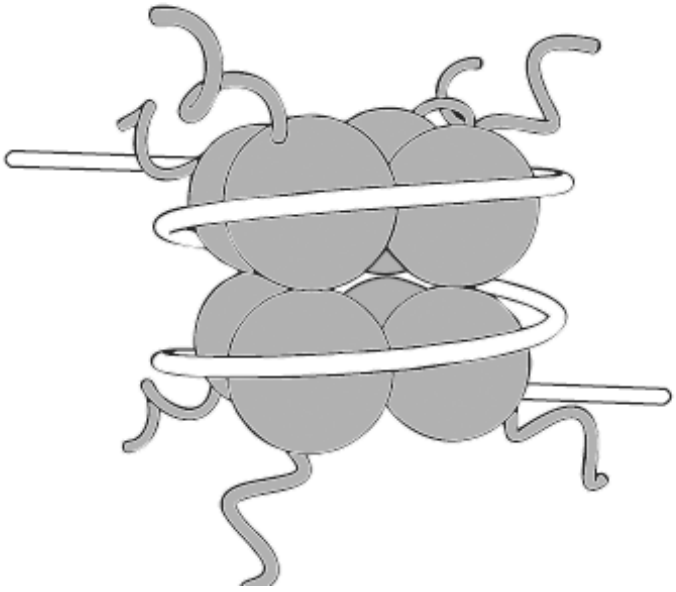

Figure 3. A simple representation of a nucleosome. The eight ball-like structures are the histones with histones tails and the white represents the DNA coiling around the octamer (Carey 2012).

Recently, it was demonstrated that histones are more than just packing proteins and play and important role in epigenetics. The degree to which DNA is bound to the nucleosome is believed to be an epigenetic process. In regions where the DNA is loosely bound to the nucleosome, the genes can actively engage in protein synthesis. In regions where the DNA Is more tightly bound is where the genes are inactive (Francis 2011). There are some regions of the chromosomes which maintain the nucleosome structure almost all the time and these regions tend to be areas of the chromosome that don't really code for any genes. This is because in regions where the DNA is tightly coiled on the nucleosome, genes cannot be accessed for replication (Carey 2012).

Histones are also extremely important in areas where the chromosomes are not always tightly coiled in this way. The histone tails can be modified in several ways, but the two most studied and understood in relation to epigenetics are histone acetylation and methylation (Tammen 2013). Acetylation is the process in which acetyl groups (-$COCH_3$)

are added to the histone tails. A class of enzymes called Histone acetyltranferases (HATs) transfers the acetyl groups onto the lysine on the histone tail. When the acetyl group is added, the positive charge on the lysine group is neutralized and this neutralization of charge weakens the bond between the histone tail and DNA. This loosens the DNA from the nucleosome and allows for transcription of that section of the genome to occur. The process of histone acetylation can be reversed by histone deacetylases (HDACs) that are able to remove the acetyl group from the lysine and restore the positive charge. HATs lead to the activation of transcription, where HDACs lead to repression of transcription (Tammen 2013).

While acetylation is specific to lysine on the histone tail, methylation can occur on several different locations of the histone tail. Whether or not the methylation of the histone tail leads to repressions or activation of transcription depends on where the methyl group is added. Methylation is able to alter transcription through the recruitment of different chromatin factors. Methyl groups in different locations are responsible for specific recruitment of proteins, which leads to a histone modification affecting the transcription of genes (Tammen 2013).

DNA methylation and histone modifications are the main mechanisms of epigenetics, but they are not the only ones. There is increasing research that demonstrates that both chromatin remodeling and microRNAs are also able to impact gene transcription. However, since these two categories of epigenetic mechanisms are more minor, I will not go into detail about the actual processes (Tammen 2013).

So far I have mainly focused on how epigenetics is important to normal development of an organism. However, these mechanisms that affect gene transcription do

much more than just guarantee that our differentiated cells remain differentiated throughout our lifetime, and that these codes are passed onto our offspring. Epigenetics is a field that goes beyond questions of normal development and looks to answers many different aspects about how the environment can affect our genes. Research in epigenetics has explored topics from diet, to stress, to use of substances like alcohol and tobacco. From this research, we know that many different factors contribute to writing our epigenetic code. Through these stable and heritable mechanisms discussed above, things that happen to us during our lifetimes can be "remembered" on our genome. Not only will these "genomic memories" affect us during our lifetimes, it is possible for these affects to be passed onto future generations.

The power of mothers

The first step to understanding how our environment changes our epigenetic code is to understand the lasting effects that changes in our parents' environments can have on our development. Conceptually, it is much easier to accept that changes to the mother's environment and experiences affect fetal development in ways that could have drastic impacts throughout our lives than how the father's environment could have lasting impact. The mother is the fetus' sole environment while developing and different studies have found that anything that happens to the mother, whether it be a change in diet, substance intake, or even stress, could create changes in our development and outcomes. These changes are not always obvious and it could take many years for to them to become clear in the offspring. Beyond that, a single change to the mother's environment could lead to multiple changes in the fetus and put the fetus at higher risks for certain health and mental conditions as adults. Timing is also critical in determining the effects environmental factors and depending on when a fetus is affected during gestation, there could be drastically different changes in the fetus as a response to the environmental factor. There are a lot of factors that contribute to the "programming" of a fetus and a real world example that points to the complexity of the issue at hand is the Dutch Hunger Winter.

The Dutch Hunger Winter occurred during World War 2 from 1944-1945. To punish the Dutch from their resistance to the Nazi Party, Nazi authorities imposed extreme food rationing in the Western Netherlands. During this time period, the caloric intake of the typical citizen was restricted from 1800 calories to 600 calories a day. This extreme restriction of food lasted for 7 months, until the Dutch liberation by the Allied forces, after which the food intake of the Dutch returned to normal (Gilbert and Epel 2009). Amazingly

enough, during this time period of extreme deprivation, children were still conceived and born. Obviously, this isn't the only major famine that has ever happened, but this famine is unique because we know the exact date that it started and ended. This, combined with the medical records kept by the Dutch, means the pregnancies and births that occurred during the time period could be traced. By tracing these people, we can see how the famine affected these individuals throughout their lives and whether the impact of the famine differed depending on when in gestation a fetus was exposed (Gilbert and Epel 2009). Many researchers have taken advantage of the unique opportunity to study the effects of this horrible time in history and the results of the multiple studies create a compelling picture of the serious impact this famine had on the generation who experienced its effects before their lives even began.

Some of the first studies done on the Dutch Hunger Winter examined the birth weights of offspring born and conceived during the famine. Birth weights can be important indicators of future health, where infants with low birth rates may be more at risk for many different health problems, and can even suffer from more long-term problems, such as social problems and delayed motor development ("Low Birthrates and the Environment"). Using the Dutch records, researchers found a trend that infants whose mothers' experienced normal nutrition levels during early gestation, but who were affected by the famine during late gestation, tended to be born small compared to normal birth weighs. However, pregnancies that were affected during the early part of development and whose mothers' regained normal caloric intake towards the end of the pregnancy tended to be born at normal birth weight. (Gilbert and Epel 2009).

When the studies on birth weights were expanded to include data on the offspring for decades after birth, there continued to be differences between the babies who were born at different weights. These additional studies found a connection between the birth weights of the babies and their adult weight. The babies who were born with a low birth weight were more likely to remain small their entire life. This group of offspring had obesity rates that were much lower than the national average. However, the offspring who were affected by the famine during early gestation but born at a normal birth weight saw the opposite effect. This group of adults had obesity rates that were significantly higher than the normal population (Painter et al. 2005).

These differences demonstrate how critical timing is when determining the effects of environmental factors during gestation. Even though these offspring were all exposed to the same famine, there are drastic differences in how this same environmental factor affected the weights of the offspring for the entirety of their lives. During development, we are able to adapt to different experiences and pressures we face while in utero. This type of developmental plasticity is known as a predictive adaptive response, or PAR (Gilbert and Epel 2009). The PAR model states that during development, an environmental cue can cause a shift in the phenotype of an offspring to match the "predicted" later environment based on the cues that occur during development. Sometimes, these predictions are very wrong and phenotypes are expressed that are not suited for later environments. This is known as the environmental mismatch hypothesis (Gilbert and Epel 2009). With regards to the Dutch Hunger Winter, it appears as those affected by the famine during the early part of gestation are "mismatched" for their actual adult environment. The malnutrition the fetus experienced in early gestation altered the phenotype of the fetus to anticipate poor

nutrition their entire lives. However, since the famine ended and the diet was returned to normal, this put these offspring at a higher risk for obesity because phenotypes that would be appropriate for low nutritional settings are not appropriate to maintain a healthy weight when food is readily available. This same mismatch was not seen in the group affected by the famine in late gestation, suggesting that the fetus was too late in development to alter their phenotype in response to the malnutrition they were experiencing (Stein et a. 2009). These results highlights how important the timing is when considering the impact of certain environmental factors are on development.

I mentioned before that a single environmental factor could lead to a whole cascade of changes in the offspring affected by them. Different cohort studies have linked the famine with different issues of mental health and adult disease beyond what I have already discussed for birth weights. One study by Hoek et al. (1998) demonstrated that those who were affected by the famine during early gestation, both males and females, had higher rates of schizophrenia as adults (Hoek et al 1998). In addition to mental health, studies have also found a link to the malnutrition during pregnancy caused by the pregnancy to risk factors for coronary heart disease. These risk factors include impaired glucose tolerance, high cholesterol, increased blood pressure and obesity. The in utero origins of these adult conditions are programmed at different times during development. This means that exposure to the famine at different times during gestation put the offspring at a greater risk level for these conditions. Additionally, this study found a link between mid gestation famine exposure and increase prevalence of obstructive airway disease (Painter et al. 2005).

While these findings from the various studies do not guarantee these medical conditions in the famine offspring as adults, combined they demonstrate the increased risks people might face if exposed to something like malnutrition during gestation. It is not likely that any of us in our lifetimes will experience a famine like what the Dutch experienced, so it is easy to wonder why these findings matter to use. While the Dutch Hunger Winter was an extreme case of famine, but even more minor variations outside of the normal range of nutrition can put a fetus at high risk for the same health conditions caused by the Dutch famine (Gilbert and Epel 2009). While famines are uncommon, malnutrition is not nearly as uncommon and this potentially makes these findings relevant to a much larger scope of people.

These studies do not directly support that exposure to environmental factors in utero cause changes in the epigenetic code, but they help explain part of the story. I stated that predictive adaptive response, a type of developmental programming, as a possible explanation for some the in utero origin of adult disease. Some mechanisms of developmental programming are epigenetic mechanisms, namely DNA methylation (Langley-Evans 2006). This means that the conditions the fetus experienced caused alterations in the methylation pattern of the genome. Alterations would have the power to drive some of the reprogramming in the offspring. Furthermore, alterations of the methylation pattern can be maintained for an entire lifetime, so the changes could endure and potentially cause problems for an individual even into adulthood. While I just explained a possible way that epigenetics could cause the changes in disease risk factors, this is basically speculation without experiments that presence of epigenetic mechanisms in the origin of in utero health conditions brought on by environmental factors.

The need for proof of epigenetic mechanisms at work requires a different type of research than what we had for the Dutch Famine cohort studies. With the famine studies, researchers were not the ones manipulating the conditions of the study and were merely taking advantage of the unique opportunity the Dutch Hunger Winter presented. But the problem with this type of study is that we are unable to control the conditions, which makes it harder to test a specific hypothesis. For what should be obvious ethical reasons, researchers cannot create studies that alleviate these concerns and maintain using a human model in their research. Even if it were ethically sound, the use of human models present unique logistical problems, such as the length of our lifespan and the ability to maintain subjects in a study, making it even harder to create an experiment to measure epigenetic mechanisms. For these reasons, epigenetic research relies heavily on the use of non-human models in experiments. In particular, many studies in epigenetics are conducted on rodents. These non-human models are incredibly important in furthering our understanding of the extent epigenetic changes influence our health.

Research on maternal epigenetic effects and its mechanisms can look effects caused both during pregnancy and from postnatal maternal care. The first several studies I will look at deal primarily with how epigenetic changes can during pregnancy.

A review by Knopik et al. in 2012 examined the epigenetics of maternal cigarette smoking during pregnancy and its effects on child development. Its common knowledge that women shouldn't smoke during pregnancy, but unfortunately, many women still take their chances with it. Through their review, they examine the different postnatal health issues and conditions that are associated to prenatal smoke exposure. Beyond that, they explore the mechanisms through which these changes occur in the fetus. It turns out that

based on different studies, epigenetics plays a role. Prenatal smoking exposure is associated with a number of serious health conditions. There are neurodevelopmental and behavioral consequences as a result of smoke exposure in infants, children, and adolescents. The consequences of smoking on neurological development were vast and ranged from intellectual impairment in children whose mothers smoked 10 or more cigarettes per day while pregnant to decreased auditory brainstem response latency (Knopik et al. 2012). Smoking has also been linked to many other problems such as asthma and allergies, cancer later in life and placental complications, among other things.

The research reviewed by Knopik et al. collectively points to the importance of DNA methylation in the process of developmental programming that leads to health problems in the offspring. One study included in the review by Knopik was a study conducted by Toledo-Rodriguez et al. (2010) and discovered one of the clearest epigenetic pathways related to neurodevelopmental outcomes. In their experiment, Toledo-Rodriguez found that in utero exposure to smoking is linked to high levels of DNA methylation in the BDNF exon 6 in adolescents. The brain-derived neurotropic factor (BDNF) is important for long-term memory and is able to act on neurons in the central nervous system and the peripheral nervous system and encourage the growth and maintenance of new and existing neurons and synapses. The BNDF gene contains several exons and exon 6 appears to be especially sensitive to epigenetic modifications. These exons are critical for the proper functioning of the BDNF proteins so when methylation patterns are altered, the BDNF may alter the ultimate expression of BDNF and further down the line interfere with the normal brain development and plasticity (Toledo-Rodreiguez et al. 2010, Knopik et al. 2012).

These downstream alterations could help account for the neurological issues that are seen in adolescents whose mother smoked throughout pregnancy.

The review by Knopik et al. (2012) also points to studies that link methylation in the placenta, umbilical cord blood and maternal blood to prenatal exposure to smoking. There are several genes related to each of these three areas that have shown altered methylation patterns after in utero smoke exposure. However, these gene modifications have not been directly linked to any of the health conditions associated with prenatal smoke exposure. This does not mean that these links aren't there, it simply points to a gap in the current body of research. We know that prenatal smoking can cause altered methylation in many different genes, but more work needs to be done using animal models to discover the downstream effects of these methylation patterns. The review by Kponik et al. (2012) heavily stresses that the existing research points to the same conclusion: the need to more carefully examine maternal cigarette smoking during pregnancy in the context of epigenetics (Kponik et al. 2012).

While not a human model, studies on the Agouti mouse have demonstrated prenatal epigenetic changes caused by their mothers' diet. The Agouti mouse possesses a gene that codes for coat color, and epigenetic modifications can alter the expression of this gene. The gene can either express yellow coloration of the fur or brown coloration of the fur. During pregnancy, mother mice that are feed a diet rich in supplements, particularly folic acid, will have offspring that express the brown coloration. The folic acid is particularly important in the expression of brown fur. Folic acid has the ability to serve as a methyl donor for methylation, and when present during development, the agouti gene is methylated by these donated methyl groups. When methylated, the gene is turned off and this results in the

expression of brown fur (Dolinoy 2008). However, when a mothers' diet lacks folic acid, there is no donation of methyl groups to methylate the *Agouti* gene. When the gene remains unmethylated, the mouse will have yellow fur. It is possible to have intermediate colors as well, depending on the levels of methylation of the gene (Figure 4).

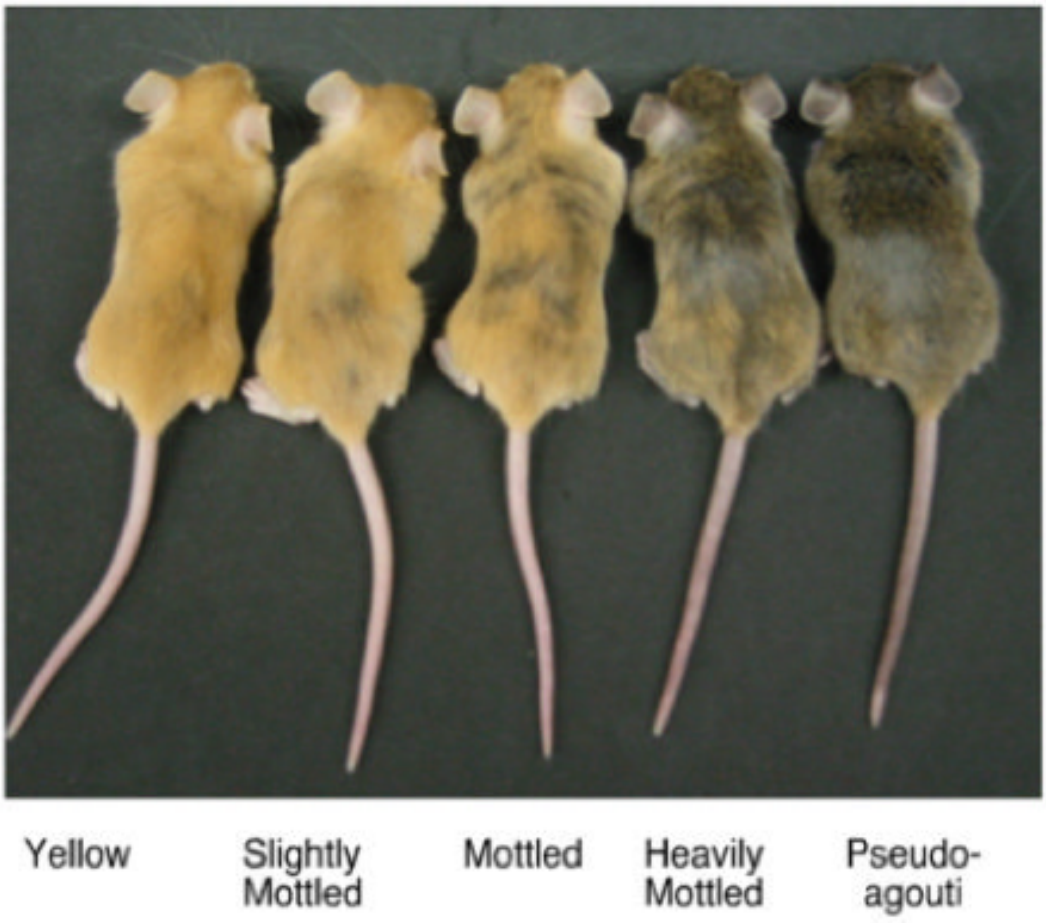

Figure 4. The different coat color types possible in the agouti mouse. The yellow mouse has no methylation on the *Agouti* gene (Dolinoy 2008).

However, the appearance of these yellow mice goes beyond a difference in fur color. This study demonstrates that it is possible for a mothers' malnutrition during pregnancy to

cause epigenetic modifications that show some of the same significant risk factors we saw in the Dutch Famine Cohort study.

While I haven't presented a completely comprehensive list of all the ways prenatal experience can affect the epigenome, the last area I will focus on is how prenatal stress affects offspring before moving into postnatal experiences. Prenatal stress is associated with increased levels vulnerability to different neurodevelopmental disorders, including autism and schizophrenia (Hodes 2013). To further test the effects of prenatal stress on offspring, Mueller and Bale (2008) performed an experiment that used mice to test the critical time period for the development of predisposition for psychological disease in a fetus. They tested the adult offspring of mice whose mothers were exposed to stress during early, mid, and late gestation. They found that the male offspring exposed to stress during early gestation had increased displays of maladaptive behavioral stress-responsivity. They also displayed anhedonia, or the inability to experience pleasure from activities normally found enjoyable, as well as increased sensitivity to Selective serotonin reuptake inhibitor (SSRI) treatments. The results of the study indicated that the increased stress sensitivity in males was likely caused by their by the altered gene expression of the glucocorticoid receptor (GR) and the central corticotropin-releasing factor (CRF). The alteration of expression of these genes is correlated with changes in the gene methylation in GR and CRF, giving evidence for an epigenetic mechanism for the changes in vulnerability in male offspring (Mueller and Bale 2008).

This study also found placental epigenetic machinery related to basal sex-difference that supports the idea that sex-specific programming begins very early in gestation. When exposed to prenatal stress, a female's placenta when pregnant with a male offspring has

increased expression of certain genes. The increased expression of these genes was seen in placentas containing male offspring but not in placentas containing female offspring (Mueller and Bale 2008). Overall, the results of this study show that stress experiences early in pregnancy may have impacts on placental function and fetal development, and this leads to male neurodevelopmental disorders.

In humans, a study by Radtke et al. (2011) examined how prenatal stress of the mother caused by intimate partner violence (IPV) would alter the methylation patters of the glucocorticoid receptor (GR) that was mentioned in the previous study. While this study is not the first to link prenatal stress to altered epigenetic methylation patterns of the GR, it was unique in the fact that it tested the longevity of these alterations. The researchers examined the methylation patterns of the GR of offspring 10 to 19 years after birth. They also looked at the GR methylation patterns of the mothers to see if the GR of mothers exposed to IPV was altered in any way. They found that the methylation pattern of the GR in mothers exposed to IPV was not altered from mothers not exposed to IPV. However, the offspring that whose mothers experienced IPV while pregnant had a varied methylation patter of their GR after many years. This means that the patterns of methylation established while in utero are in fact maintained well after infancy and into adulthood. This furthers the body of research that suggests these methylation patterns as a plausible way for prenatal stress to program psychosocial problems in adults (Radtke et al. 2011).

Maternal epigenetic effects are far from limited to prenatal experiences. In fact, there are many studies that explore the influence a mother has on altering the epigenetic code of her offspring through postpartum. The level and quality of maternal care in both human and nonhuman models is proving to play a much larger role in an offspring's

epigenetic code than origin expected. These effects have even been linked to transgenerational effects, meaning the effects are maintained for multiple generations.

In humans, there are several examples of early infant environment leading to lasting effects into adulthood. Epidemiological studies have demonstrated the importance of family function and early life events as possible predicators of adult health (Meany 2010). Negative early life events, such as being a victim of childhood physical or sexual abuse, experiencing harsh and inconsistent discipline, and emotion neglect is linked to negative health outcomes in adults. These early life events put the individuals who experience them at a much greater risk for mental illness, obesity, gastrointestinal illness and heart disease. Models, called Stress diathesis models, are proposed as explanations for this type of relationship between early experience and health later in life. Stress diathesis suggests that being faced with adverse conditions during early life alters the development of certain neural and endocrine responses to stress. These alterations may occur in a manner that predisposes individuals to disease (Meaney 2010).

When an individual is stressed, it is logical that the stress-defense mechanisms will be activated. These stress-induced mechanisms are normally defined by the increase in the synthesis and release of glucocorticoids and catecholamines. The persistent over production of these defensive responses lead comes at a cost. Unfortunately, this cost is that the body's response to this chronic production is what sets up individuals for a predisposition for health issues as they age. This theory of Stress Diathesis is an epidemiological theory that demonstrates some of a possible link between early life stress and adult disease, it does not provide us with the epigenetic mechanisms for how these changes arise (Meaney 2010).

The only way to find this "missing link" of a mechanism is through experiments that look for changes in the epigenetic code in individuals who experience early life stress. One such experiment was a study conducted by McGowan et. al (2011) that examined the possibility of epigenetic regulation in human brains of those who experienced child abuse. In their study, they compared a neuron-specific glucocorticoid receptor, *NR3C1*, promoter region between the postmortem hippocampus of suicide victims with a history of child abuse to suicide victims to victims without a history of child abuse and a control group. They discovered some very notable differences between the three groups. In the suicide group with a history of child abuse, there was a decrease in the hippocampal *NR3C1* gene expression (McGowan et al. 2009). The hippocampal samples also showed increased methylation of the exon 1_F *NR3C1* promoter in the suicide victims with childhood abuse (Figure 5). These patterns were not seen in the control group of the suicide group without a history of childhood abuse (McGowan et al. 2009).

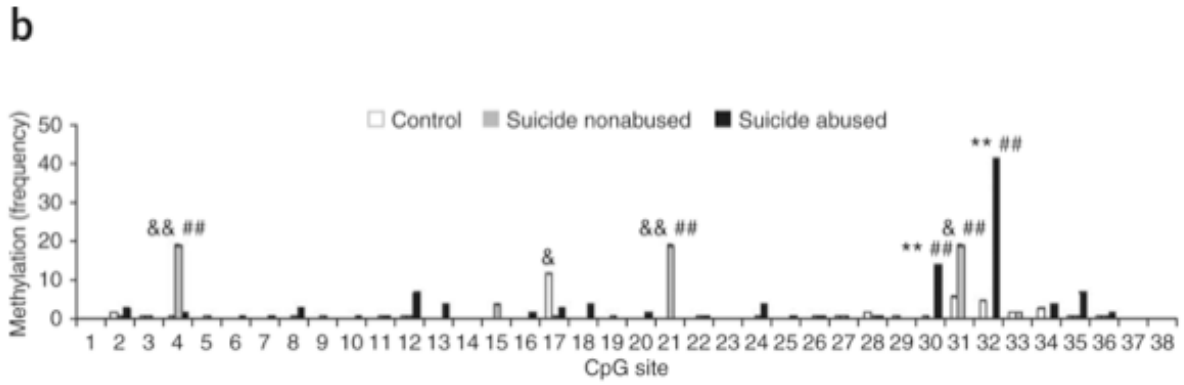

Figure 5. The methylation levels of the *NR3C1* hippocampal promoter regions (McGowan et al. 2009).

This study demonstrates an epigenetic mechanism in which the brain of children who experience abuse is actually changed, possibly making them more prone to depression and suicide than those with dissimilar childhood experiences. The authors of this study state that these results are consistent with other studies using psychological autopsy and epidemiological longitudinal designs that point to the conclusion that suicide has a developmental origin. Daily exposure to an abusive mother has also been associated with an increase in DNA methylation of the BDNF IV promoter region. This increase of methylation is associated with a decrease in the expression of BDNF in the prefrontal cortex of the adult brain (Champagne 2011).

Some of the most extensive research on maternal epigenetic effects was done using mouse and rat licking studies. A review by Champagne (2011) provides a series of studies where researchers manipulated the quality and quantity of care a rodent's pups will receive and test for epigenetic changes within the groups of pups. Research of this type really began in the late 1950s and early 1960s, when psychologists Victor Denenberg and Gig Levine found that postnatal handling of infant rodents decreased the magnitude of behavioral and hypothalamic-pituitary-adrenal (HPA) response when the mice are adults (Champagne 2011). This suggested that the handling of mice could lead to the reduction of the stress defense mechanisms used in mice, especially those who are in an early stress environment. Levine and Denenberg suggested that the differences in the seen in these mice were mediated by maternal care. When they tested this theory, they found that in fact, the increase in postnatal handling increased the licking and grooming (LG) of the pups by the mother. The LG a rodent pup receives is incredibly important because this tactile stimulation plays a role in regulating the endocrine and cardiovascular system in the pup. A

large part of typical maternal behavior in rodents in the amount of LG they provide to their pups. However, the changes in maternal behavior (increased/decreased LG) can be induced very rapidly depending on changes in the postpartum environment (Champagne 2011). The combination of the importance of LG on the pups and the maternal behavior being so easy altered based on environment has made this a very efficient way to study epigenetics and helps explain why many studies in the field have taken advantage of these behaviors in their studies. While these studies are extremely abundant, I will only include a few in this paper to represent some of the knowledge we have gained from them.

The first step for this type of research was to determine in the quality of mother-infant interactions during the development will alter epigenetic marks in a way that will lead to individual differences in the offspring. Several studies demonstrated that there is a link between maternal care (high LG and low LG dams) and the differential expression of ERα in the MPOA of female offspring. Messing with the expression of the ERα, or the estrogen receptor α, is linked to a lack of sensitivity to priming events during development (Champagne 2011). The compilation of studies that examined the 1b promoter region of the ERα gene, found similar results in that there are several CpG sites in this area that could potentially be methylated. Methylation in this region would lead to reduced ERα mRNA. In offspring who were reared by low LG dams, there was an elevated level of DNA methylation in the above mentioned promoter regions. This research demonstrates that there can be stable epigenetic changes brought on in offspring based on the maternal expressions of licking and grooming of offspring (Champagne 2011).

A different study by Hellstrom et al. (2012) looked at how variations in maternal licking and grooming and how it affects the regulation of differences in the hypothalamic-

pituitary-adrenal response to stress. They looked at offspring from mothers who displayed both an increased and decreased frequency of licking and grooming. The researchers focused on how the frequency of the maternal licking and grooming affected the hippocampal glucocorticoid receptor. The changes in epigenetic mechanisms brought on by maternal care on the hippocampal glucocorticoid receptor occur through the DNA methylation of the exon 1_7 GR promoter (Hellstrom et al. 2012). This study found that the adult offspring of mothers that show an increased frequency in licking and grooming had increased hippocampal glucocorticoid receptor expression and also exhibited a more mild pituitary-adrenal response to stress than those who had mothers who exhibited a lower frequency in LG. The increased expression of the hippocampal GR is from the maternal care is mediated by the epigenetic reprogramming caused by the binding of a transcription factor nerve growth factor-inducible factor A (NGFI-A). The interaction of the NGIF-A with the exon 1_7 GR promoter is dramatically increased in offspring that experience more LG from their mothers. This study also found that the same epigenetic reprogramming is possible through artificial touch stimulation. Adult offspring who were exposed to artificial tactile stimulation comparable to that provided normally the mothers experienced the same effects as those who had high LG mothers.

A lot of people joke as they get older, that they become more and more like their parents, and when it comes to the type of mother a woman will become, research suggests that it is highly probably they will be more like their mothers than they think. The quality of care a mother provides for her offspring is critical in development and for humans and primates and there is evidence for the matrilineal transmission of maternal behavior. Women raised in an institutional setting where their own childhood lacked parental

influence are more likely to display less sensitivity and be more confrontational to their own children. An important part of epigenetic research is how environmental influences have the potential to not only affect the direct offspring of the those who experience them, but also generations beyond that. A lot of research in transgenerational epigenetics has focused on epigenetic changes that are passed through the maternal line.

The study I previously mentioned on the ERα expression in the MPOA is a study that was expanded to include transgenerational research. The study found that the epigenetic changes caused by maternal care are actually transmitted across generations of female offspring. The methylation patterns of the offspring alters the type of maternal care that offspring will provide to be similar to the care the mother gives. This means their offspring will experience the same type of care and once again have the same epigenetic reprogramming occur. Figure 6 is a visual representation of the cyclic patterns demonstrating the multigenerational effects (Champagne 2008).

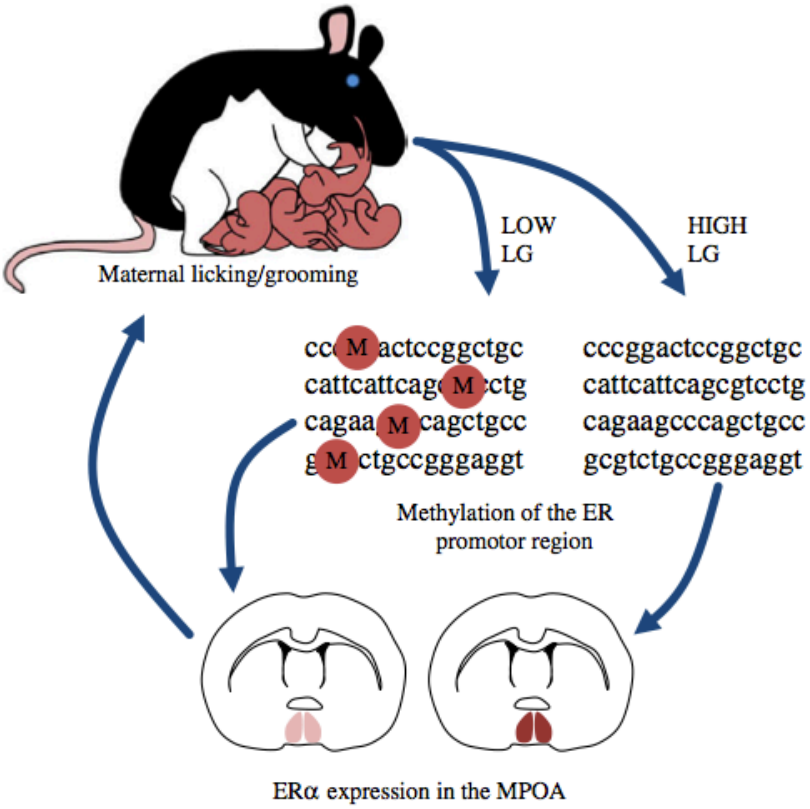

Figure 6. Pictorial demonstration of how the type of care the mother provides her offspring can begin a cycle of care and methylation patterns (Champagne 2008).

This is not the only study that shows that maternal behavior can actually be inherited by future generations through nongenomic means. For instance, fearful mothers tend to be mothers who display a decreased frequency of LG to their pups (Meaney 2010). These fearful mothers tend to raise offspring that are more stress reactive themselves. As the previous studies in this paper have demonstrated, we know that the postnatal environment provided to offspring can have a powerful affect on the epigenome and phenotypic expression. The studies on fearful mothers show that female offspring of the fearful, low LG mothers also tend to be fearful, low LG mothers when they reproduce. In contrast, mothers that display high LG frequency towards their offspring tend to have female offspring that are high LG themselves when they reproduce. This trend would lead

to a continuation of this pattern of maternal care for multiple generations and the continuation and maintenance of the altered epigenetic pattern (Meaney 2010).

A study done by Burdge et al. (2007) demonstrated how the epigenetic code is altered when one part of a rat's diet is restricted. In this experiment, the researchers tested the hypothesis that effect of feeding a rat a protein restricted diet would result in altered genetic expression in first and second-generation offspring. Feeding a reduced protein diet (PRD) during pregnancy was previously linked to an alteration in the metabolic phenotype of the first and second generation of offspring. This induced alteration of metabolic pathways in the liver of the rat is caused by a change in the methylation of specific gene promoters. In this case, the gene promoters that are altered are the peroxisomal proliferator-activated receptor (PPARα) and the glucocorticoid receptor (GR) promoters. The results showed that in when the mother is feed a PRD, the first and second generations of rats had significantly lower Hepatic PPARα and GR promoter methylation than normal. The second-generation mothers were fed normal diets without protein restriction, so the methylation patterns were somehow passed down for multiple generations (Burdge et al. 2007).

These studies demonstrate that it is possible for an epigenetic modification to make a very lasting impact. Different environmental experiences can contribute to changes in the epigenome for several generations to come. However, while these modifications look extremely stable, not all epigenetic modifications are that way. As I discussed in the beginning section of this paper, methylation is a reversible mechanism. While it has the ability to be maintained and is stable within an organism to the point where acquired characteristics can sometimes be inherited, it does not have to be a permanent alteration to

the genome. There is a lot of research being done within epigenetics to discover how epigenetic changes can be reversed within an organism. I am not going to go into detail about the current research being done in that area, but the fact that this research is occurring is a good reminder that epigenetic changes are reversible.

A Father's Contribution

While maternal epigenetic effects can be readily explained by the interactions between the mother and the fetus and also the mother and the care of her offspring, it is not as simple to draw a link for paternal epigenetic effects. It's not only the fact that it is hard to find the link between the father and the fetus to create these effects, but it was believed that that father's sperm didn't contribute anything besides genomic information. It was also believed that the genomic information was relatively safe from environmental effects and the father was unlike to pass on epigenetic effect. Expanding on the research demonstrating the epigenetic effects between the mother and the fetus, researchers are discovering that the father can pass down more than just genomic information. The DNA methylation patterns and histone modifications that are built up during a father's lifetime might not be "wiped clean" off the genome during the creation of sperm and these modifications can be carried with the genome for the creation of the zygote. However, the research into paternal epigenetic effects began in recent years (Rando 2012). This means that while we have a considerable amount of research on maternal epigenetic effects, there is not nearly the same body of work when it comes to paternal epigenetic effects. However, there are studies that have not only demonstrated paternal epigenetic effects, there are even studies that show transgenerational paternal effects.

Food intake and nutrition is an important factor in creating epigenetic changes in the paternal line. Research has found that there are transgenerational effects on the phenotypes of sons and grandsons of males with altered food intake during critical time periods of their lives. Three different studies demonstrated that changes in early life programming and nutritional experiences of fathers and grandfathers could increase or

decrease adulthood risk for disease (Kaati et al. 2002, Kaati et al. 2007, Pembrey et al. 2006). These studies point to the slow growth period of prepubescent adolescents as a critical period of development. The slow growth period is usually longer in boys than girls and occurs in girls from about age 8-10 and boys from 9-12. During this slow growth period, it appears that organisms are especially susceptible to changes in themselves and offspring from variations in food availability.

In these studies, the researchers followed extremely similar procedures. In each of these studies, the researchers gathered information from individuals of three different cohorts who were born between the late 19th and early 20th century. Because the differences in the ages, each cohort had a separate set of nutritional experiences and their ancestors also had different nutritional experiences, particularly during their slow growth period. The term proband is used to describe the individual being studied through the use of their medical records as well as the medical records of their parents and grandparents. The studies compared the health status and causes of death of probands to the nutritional experiences of their parents and grandparents.

In the study performed by Kaati et al. (2002), they attempted to answer the question of whether overeating by a child during their slow growth period would influence their descendants' risk of death related to cardiovascular disease and diabetes (Kaati et al. 2002). The parents' and grandparents' food access was assessed using historical records to determine periods of famine and good harvest during each of their slow growth periods. The study found paternally linked transgenerational responses caused by the variation in food intake. When the father of a proband was exposed to limited food access during their slow growth period, the probands were at low risk for cardiovascular disease related

death. Also, if the paternal grandfather lived through a famine, this appeared to protect the proband from diabetes. However, if the paternal grandfather of the proband had an abundance of food during their slow growth period, the proband was four times greater risk death from diabetes. While these results demonstrate that sex-specific responses occur from nutritional variation, there is no way to demonstrate the actual epigenetic mechanism that could create these changes. While the authors believe that epigenetics is the most probable explanation for these results, they state that there is too little known to speculate how nutritional variation leads to alterations in the male germline (Kaati et al. 2002).

The study by Pembrey et al. (2006) was to detect transgenerational effects through the male line based on different environmental factors. While this study did not exclusively look at nutritional factors, a large part of the study was performed using the information of probands and their parents and grandparents and determining their food intake during their slow growth period following the previously described methods. Their results did not find an impact on either gender grandchild's relative mortality risk ratio from due to the food supply of their maternal grandparent. They did find that there is a sex-specific relative mortality risk ratio increase when the dependent on the food intake of the paternal grandparents. If the paternal grandfather experienced good food supply during their slow growth period, there was an increase in the male proband's relative mortality risk ratio compared to probands whose grandfathers had different nutritional experiences during their slow growth phase. The opposite effect was observed in male probands when their paternal grandparents experienced low food availability, with there being a reduction in the male probands relative mortality risk ratio (Pembrey et al. 2006).

There were also changes based on the paternal grandmother's food access during her slow growth period, and these changes occurred in the female probands only. When the paternal grandmother experienced good food availability during her slow growth period, the female probands had double the mortality risk ratio as those whose paternal grandmothers had other experiences. Also, when the paternal grandmother experienced low food availability during her slow growth period, the female proband has a reduced mortality risk ratio. The results of this study demonstrate similar changes for paternal grandfather to grandson and paternal grandmother to granddaughter, but the changes are sex-linked, so changes to the grandfathers food intake during his slow growth period had no effect on the granddaughter and vice versa. Because of these sex-linked transgenerational effects in the paternal line only, Pembrey et al. suggest that these effects are mediated by the x and y chromosomes through epigenetic regulation (Pembrey et al. 2006).

The last study of that followed the same basic procedure was a study done by Kaati et al. in 2007. This study, among other things, looked at once again how food availability during ancestor's slow growth period affected the proband's longevity and mortality risk. The results of this study further strengthened the findings of the previous two studies in demonstrating once again there is a sex-linked paternal transmission of environmental effects. Once again, the altered food availability for the paternal grandparents caused health alterations in the same-sex grandchild. This study also demonstrated that when conditions of the proband's upbringing were controlled for, male probands whose fathers had good nutrition during their slow growth period had an increased risk of mortality.

Kaati et al. (2007) provide three reasons for why epigenetics is a candidate to

explain all these transgenerational effects. The reasons he states are that previous research has demonstrated that epigenetic modifications are transmitted through generations, nutritional exposures can alter the epigenetic code, and that transgenerational responses in both humans and animals can occur from specific exposures. A complete explanation for the results of the previous three studies would require a combination of the three points to completely support epigenetic causes as the cause of these effects. However, at the time this paper was published, there were very few existing examples in existing research that combine these three factors(Kaati et al. 2007).

A study done by Braunschweig et al. (2012) attempted to solve the ambiguity surrounding the existence of epigenetic mechanism as the cause of the transgenerational effects. Braunshweig et al. designed this pilot pig study based on the studies by Kaati et al in 2002, Pembrey et al., and Kaati et al in 2007 as well as the research on the agouti mice that I discussed in the maternal effects section. Researchers fed the experimental pig group a standard diet supplemented with high amounts of methylating micronutrients. The supplemental diet of highly methylating micronutrients was similar to the diet that was fed to the pregnant agouti mice that induced the epigenetic fur color change. They hypothesized that the diet would cause epigenetic alterations to the boar's offspring's genomes and based on the studies by Kaati et al in 2002, Pembrfey et al., and Kaati et al in 2007, they hypothesized that any changes to the epigenome would be maintained for two generations through the paternal line. To determine the differences in the boars brought about by the altered diet, they measured changes in physical characteristics of the boar's carcass, gene expression profiles, and DNA methylation patterns (Braunschweig et al. 2012).

The analysis of the results showed that both the first and second-generation offspring of the experimental group were overall leaner than the first and second-generation offspring of the control group. When the gene expression profiles were examined, the profiles showed significant differences in the mRNA levels between the experimental group offspring and the control group offspring. The altered gene expression affected gene 79 in muscles RNA, gene 64 in liver RNA, and gene 53 in kidney RNA. In the experimental group, the differential expressions of the genes lead to an increase in the respective pathways for lipid metabolism and metabolic pathways in the liver and muscle. The final differences they observed was found in the promoter regions of the differentially expressed gene and found differences in DNA methylation of the promoter regions in the *IYD* gene. These differences in methylation could interfere with the transcription-binding factor, resulting in changes in the organism (Braunschweig et al. 2012).

The combination of these results demonstrates that there are mechanism changes that could be epigenetic in nature. However, this study alone wasn't able to completely separate what of the observed changes were genetic or epigenetic in nature. Additionally, with respect to the DNA methylation analysis, the researchers were only able to analyze the methylation patterns of six different genes. The researchers state that the limited analysis of promoter regions likely means only a limited amount of methylation changes were detected. If the analysis were done on a greater scale, this could help differentiate between what changes are truly epigenetic in nature and what aren't (Braunschweig et al. 2012).

While nutritional variations in the paternal line has the potential to have transgenerational effects, the father's diet and nutritional status could have other epigenetic impacts as well. Obesity is typically linked to over-nutrition, unbalanced food

intake and a sedentary lifestyle, factors that could lead to epigenetic effects. In a 2013 study by Soubry et al., the researchers looked at how paternal obesity (having a BMI ≥ 30) could have a preconception impact on the methylation patterns of offspring. The researchers analyzed DNA from umbilical cord blood leukocytes and compared the DNA and methylation profiles to the medical records of the mother and father of the newborns. The analysis of the DNA methylation patterns focused on the *Insulin-Like Growth Factor 2 (IGF2)* gene because this gene is a well-characterized growth factor that is active throughout development. When the paternal *IGF2* allele is transcribed, there are two regions that differentially methylated region (DMR) and subject to imprinting. These differentially methylated regions are the *IGF2* DMR and the neighboring non-coding *H19* DMR. After determining the levels of methylation at the CpG sites in the *IFG2* and *H19* DMR, researchers found that in the *IFG2* DMR of newborns who have obese fathers, there was significantly less methylation on the CpG sites. In the *H19* DMR there was no difference between newborns with non-obese and obese fathers in the amount of methylation on the CpG sites (Soubry et al. 2013).

These results suggest that paternal obesity does in fact have the ability to impact offspring's epigenome. Existing research suggests that methylation at the *IFG2* site in sperm is controlled by levels of estrogen. Adipocytes, fat cells, can produce estrogen, meaning an increase in adipocytes in the obese fathers would increase levels of estrogen. The increased exposure to estrogen might disrupt the functioning of epigenetic mechanisms and lead to the inability to make the proper methylation marks during spermatogenesis. Spermatogenesis is the production of sperm and since this process occurs throughout an adult male's life, the process is sensitive to the weight of the father.

This inability to establish the proper markings in sperm would lead to differences in the information being passed onto offspring. Previous studies have shown that differences in the methylation of the *IGF2* DMR are associated with higher levels of IGF2 in the offspring and an increased susceptibility to chronic disease (Soubry et al. 2013). While this study had a small sample size, the authors state that this was the first study of its type. This study begins to explains some mechanism that are passed through the paternal line, but further studies are needed with expanded sample sizes and that examine the methylation patterns of genes to expand knowledge on paternal obesity and its effects on offspring.

Beyond the influence paternal nutrition has across multiple generations, a variety of studies have demonstrated that paternal effects can arise through exposure to drugs and toxins. The study by Pembrey et al. in 2006 that demonstrated sex-specific transgenerational paternal effects from nutrition also looked at paternal smoking could lead to transgenerational effects. Using the same sample of individuals from the nutritional study, researchers compared the smoking histories of the fathers to the health status of the probands. The goal was to determine the impact of the fathers smoking history and the age the father smoking on the BMI of the proband. The analysis revealed that the BMIs of male and female probands at age 9 increased as the age their fathers began smoking decreased (Table 1).

Table 1 Adjusted mean (SE) BMI by age and sex against age of onset of paternal smoking

	Boys		*Girls*	
Age father started smoking	*Age 7*	*Age 9*	*Age 7*	*Age 9*
<10	16.53 (0.33)	18.15 (0.55)	16.45 (0.37)	18.64 (0.55)
11–12	16.22 (0.23)	17.73 (0.35)	16.33 (0.23)	18.22 (0.32)
13–14	16.07 (0.14)	17.75 (0.21)	16.54 (0.16)	18.24 (0.23)
15+/never	15.98 (0.08)	17.23 (0.12)	16.41 (0.09)	18.04 (0.12)

Table 1. The data for adjusted mean BMI of male and female probands depending on the age their fathers began smoking (Pembrey et al. 2006).

The researchers also compared the father smoking habits at the time of conception to the BMI of the probands. The results showed that if the father smoked during the time of conception, the male probands had significantly higher BMIs than the male probands whose fathers did not smoke at the time of conception. However, this difference was not observed in the female probands (Pembrey et al. 2006).

A study by Hillemacher et al. (2007) examined the effects of paternal smoking on DNA methylation patterns. In this particular study, the researchers looked at alterations on global DNA methylation instead of gene-specific methylation patterns. The analysis of the data showed that the strongest associated between offspring's and paternal global DNA methylation is strongest if both the offspring and father are non-smokers. While less pronounced, there is still a significant association between the global DNA methylation patterns if the father is a smoker and the offspring are non-smokers. However, the results demonstrated that if the offspring smoked at any point in their lives, the association was lost completely (Table 2).

Table 2 Heredity of global DNA methylation in relation to smoking status

Offspring smoking status	Paternal smoking status	R^2 corrected	Beta	p
Smokers	Smokers	−0.00	0.15	n.s.
	Non-smokers	−0.05	−0.24	n.s.
Non-smokers	Smokers	0.16	0.43	0.02
	Non-smokers	0.41	0.68	0.02

n.s. = not significant.

Table 2. The results of the analysis for associations between Paternal and offspring global DNA methylation patterns based on smoking status. The table shows that there is only an association in the methylation patterns when the offspring were non-smokers (Hillemacher et al. 2007).

Together, these findings suggest that smoking may have a long-term effect on global DNA methylation. The changes to the global DNA methylation patterns further our general understanding of the effects smoking can have on DNA methylation patterns, as well as the patterns of methylation are maintained between fathers and offspring. This study needs to be expanded on to look at how gene-specific methylation patterns are changed because of paternal smoking behavior. Knowing how individual genes are affected would provide a better understanding of how paternal smoking could lead to disease and health status of the offspring.

Alcohol may also play a role in creating paternal effects on offspring, with previous research already demonstrating a link between a father's drinking habits and certain outcomes in their children. One example is that paternal alcoholism is associated with reduced birth weight in offspring (Little et al. 1987). Other early studies show that children of alcoholic fathers exhibit hyperactivity and reduced cognitive performance, but only if the alcoholic father was the biological father. The correlation between cognitive performance in children and alcoholism in the biological father suggests that there is a biological link to the existence of these traits and not just because of their environment the child is raised in (Hegedus et al. 1984). Later laboratory studies have demonstrated paternally induced effects in offspring through the father's preconception alcohol consumption. In a 2004, Ernest Abel looked at the paternal contribution to fetal alcohol syndrome. This study looked at three mechanisms through which paternal alcoholism influences offspring. They described these mechanisms as non-genetic, genetic and epigenetic. For the purpose of this paper, I focused on the section of the study that looked at epigenetic mechanisms (Abel 2004).

Alcohol was shown to have altering effects on the DNA methylation patterns of many different parts of the human body, including the liver, colon, esophagus, and uterus. When consumed, alcohol is distributed to the testes, so it has the potential to create DNA methylation pattern changes in the testes, particularly on the sperm. If alcohol is inducing DNA methylation changes on the sperm, it is possible that these changes are passed down to future generations by changing the expression of developmentally active genes in the offspring (Abel 2004). To test this hypothesis, Abel used male rats that were treated with alcohol. The alcohol was administered to the rats for 9 weeks, which is the amount of time it takes for an adult male rat to complete an entire cycle of spermatogenesis. Using sperm samples from the rats, he analyzed the levels of cytosine specific DNA methyltransferase transcription. Remember that DNA methyltransferase is the family of enzymes that are responsible for the addition of methyl groups to the DNA (Abel 2004).

The analysis of the cytosine methyltransferase showed that in the male rats who were treated with alcohol had significantly lower levels of cytosine methyltransferase mRNA levels compared to the control group (Abel 2004). The lower levels of methylation support the hypothesis that alcohol can have an effect on DNA methylation patterns of the sperm. With these levels of hypomethylation, it is possible that these changes can be passed onto offspring. Further studies are needed to see if these altered DNA methylation patterns are passed onto the offspring and to determine the how great the biological impact of a father's alcohol consumption is on the offspring (Abel 2004).

A study by Meek et al. (2007) went on to examine the effects paternal alcohol use has on offspring. This study focused on the effects of acute alcohol use in mice, so male mice were given alcohol 12-24 hours before mating. The offspring of these mice were

examined for developmental and behavioral differences compared to offspring of control group that were given saline instead of alcohol. The results demonstrated a variety of changes in the offspring. In the litters of pups from the alcohol-exposed fathers, fewer pups were born in and of those that were born, there were significantly more runts born, and more pups died in the first three weeks of life than in the control group. The gestational period for the pups of the alcohol-exposed pups was also significantly shorter than normal. Also, the pups of the alcohol-exposed fathers missed many of the developmental milestones that the saline-exposed pups were able to achieve at the same time period. This developmental lag affected things motor functions such as clinging, tail-pull reflex, rotation, linear movement and the ability to climb up an inclined surface.

As the pups entered into adulthood, there was still a significant amount of differences between the two groups. The pups of the alcohol-exposed fathers showed much less risk assessment behavior than the control group. In fact, when presented with a container of cat-scent, the alcohol-sired mice approached the scent much sooner, remained there longer, and approached it more often than the control group mice (Table 3).

Mean latency to risk assessment behavior (s)			
Latency to behavior (s)	Saline-sired group	Alcohol-sired group	*P* value
Stretched attention	16.7±3.13	134.36±9.36[a]	*P*<0.001
Flatback	125.43±13.3	515.96±27.84[a]	*P*<0.001
Freezing	176.7±5.01	555.6±16.17[a]	*P*<0.001
Defensive burying	48.56±4.93	514.06±27.22	*P*<0.001
Contact	385.56±20.79[a]	31.9±3.31	*P*<0.001

All values expressed as mean±standard error of the mean.

Table 3. The length of time for each group to react to the container of cat-scent, as well as the amount of time it took mice from each group to make contact. The data show that the alcohol-sired group made contact with the container much sooner and remained at the container longer than the saline-sired group (Meek et al. 2007).

There were differences in the aggressive and defensive behaviors of the mice from both groups as well. The alcohol-sired group displayed fewer fearful/defensive behaviors, including jump-escapes and tail rattling, than the control group mice. Interestingly, the alcohol-sired mice displayed more frequent aggressive behaviors, such as lateral attacks and jumps attacks. These mice were on average worse at controlling these behaviors as proven by demonstrating much shorten latencies to all aggressive behaviors as compared to the control group (Meek et al. 2007).

Paternal epigenetic effects can arise by males experiencing chronic and unpredictable stressful situations. A study by Franklin et al. (2007) examined how early-life exposure to chronic and unpredictable stressful situations caused by maternal behavior lead to changes in the DNA methylation and behaviors of the first-generation male offspring, as well as second-generation offspring. The researchers were hoping to provide evidence that within mice, transgenerational transmission of complex behavioral changes in offspring induced by early life stress during postnatal development. The researchers bred litters of mice in the lab and then subjected these pups to irregular separation from their mothers before weaning. The mother was removed from the litters at random time intervals, with the length of removal being varied. There was also a control group where the researchers allowed the pups to be reared normally. To test for transgenerational effects, the pups from both the experimental group and the control group were allowed to reproduce and were reared normally. Behaviors of both the first-generation and second-generation mice were monitored and recorded (Franklin et al. 2010).

The researchers found that the irregular separation from their mothers had effects on both the first, second and third-generation mice. The main effect that was observed

were depressive-like behaviors and altered responses to novel and aversive environments in the adult mice (Franklin et al. 2010). In the first-generation males, during a forced swim test, the male rats spent significantly more time floating, which is a sign of depression in rats. These depressive behaviors in the force swim test were only seen in the male mice and not female mice, further confirming previous results that maternal separation primarily has a negative effect on males. The depressive symptoms in the first generation mice was further confirmed by the tail suspension test, where the males had increased time spend immobile, a sign of depression in mice (Franklin et al. 2010).

In the second-generation mice, both male and female mice were tested for depressive behaviors. The second-generation female mice of the males from the experimental group showed the same increase in floating times during the forced swim test as their fathers. This suggests that the depressive symptoms in the first-generation male mice could be transmitted to their female offspring. However, when these males were bred under normal conditions, their male offspring displayed the same depressive behaviors as the first-generation males. These results suggest that effects of the early life stress leads to a very complex, sex-linked transmission of the effects (Franklin et al. 2010).

Another symptom in mice that is commonly associated with depression is anhedonia, or the inability to enjoy pleasurable stimuli. To test for anhedonia in the mice, they tested the amount of sucrose a mouse would consume when presented with it. Mice who consumed less sucrose overall were considered positive for the trait anhedonia. The results of this test were consistent with the previous results. In the first generation of offspring, only the male mice decreased their sucrose intake. However, the same transgenerational effects were not seen for anhedonia, so no mice in either the second- or

third-generation offspring had a reduction in the amount of sucrose they consumed. This suggests that anhedonia is only brought on by early stress and is not passed on through generations, highlighting the idea that only certain depressive behaviors have a transgenerational link (Franklin et al. 2010).

This study also tested how the stress sensitivity in the mice was altered based on the rearing conditions because stress sensitivity is a known precursor to depression. Placing mice from the control and experimental group in conditions that were unfamiliar or averse environments assessed stress sensitivity. When exposed to a mildly stressful situation, the first-generation experimental male mice has shorter latency to enter the unfamiliar areas of the environment that the control mice. When placed in an even more stressful situation, the same male mice were more likely than the control mice to enter in the center of the stressful environment. When these traits were examined as to whether or not they were transmitted to offspring, the results were similar to that of the previous depressive-traits. The results showed that the second-generation females, not males, displayed the same characteristics as their fathers. Also, in the third generation mice, there was a level of latency that was comparable to both the first- and second-generation mice (Franklin et al. 2010).

This study was particularly powerful because unlike other paternal studies that I have looked at that either focused on the epigenetic mechanism *or* the transgenerational effects, this study attempted to do both. The authors of this paper attempted to find the epigenetic mechanism responsible for inducing the behavioral changes across the generations of mice. Because there was a sex-linked nature to the results, the researchers hypothesized that the mechanism responsible for these traits was an alteration in the male

germline. The rational of the male germline causing transgenerational behavioral alterations is because these alterations occurred independent of maternal care, so the effects between generations must have a cellular link. The authors hypothesized that the changes in the offspring were caused by DNA methylation changes in the sperm of the first generation males who experienced the maternal separation and that these changes in the epigenetic pattern were maintained through generations (Franklin et al. 2010).

To test this hypothesis, the researchers examined the levels of methylation on the promoter regions of several genes. The genes they tested were known to be involved in the epigenetic regulation of gene expression or had known associations with depression or emotional behaviors. To test the methylation patterns, they extracted genomic DNA from the first-generation male germ cells and analyzed the methylation patterns. They found that the CpG islands surrounding the transcription initiation site of the promoters of multiple genes had varying levels of methylation. In some genes, the methylation of the CpG islands was increased, while in other genes the CpG islands had decreased methylation. These results demonstrate that when a male is subjected to early-life stress this can lead to epigenetic changes to the male germ cells (Franklin et al. 2010).

The researchers then looked to see if the changes in the methylation patterns of the CpG islands were maintained across generations. The previous studies had shown that the second-generation female mice exhibited the same depressive symptoms as their fathers, so they focused the DNA methylation analysis on the female offspring by analyzing the methylation patterns of the chosen genes in the brain. The analysis showed that these females had the same methylation patters, whether hypermethylation or hypomethylation, on the same genes as their fathers. They also found that the genes that had altered

methylation patterns experienced altered gene expression that would account for the changes in behavior. The sperm of the second-generation males was analyzed to see if methylation patterns were altered, which would explain the transmission of some traits to third-generation offspring. The analysis showed that some of the genes in the sperm were in fact methylated in the same way as the first-generation males. However, there were some genes that did not have a methylation pattern that was altered in any way from the control group. This accounts for the fact that the third-generation male rats only exhibited some of the depressive behaviors of their predecessors because some of the epigenetic changes that caused the behavior change were lost over the multiple generations (Franklin et al. 2010).

The collection of studies I included in this section demonstrates that there are a wide variety of paternal epigenetic effects. However, there are many more studies that further demonstrate paternal epigenetic effects. The research available shows that while there is still an inequity between the amount of existing research on maternal and paternal epigenetics effects, the paternal research is quickly expanding. Not only is it expanding, but also the existing research shows that fathers do play an important role in transmitting epigenetic effects and we need to keep working to understand the true impact fathers have.

The Big Picture: Epigenetics and Public Health

While the body of research in the field of epigenetics is quickly expanding, epigenetics is still a relatively new field. As with any new field, it is impossible to know exactly how the information it provides us will impact the disciplines outside the field itself. The studies I presented in this paper is by no means an exhaustive list, but even these select studies illustrate the breadth of impact and understanding epigenetic research could provide.

At the advent of epigenetics, the field was intended as an explanation of the interconnectedness of development and genetics, but some of the new, developing research lends itself to applications at a much larger scale. In the collection of studies I looked at for this thesis, I looked for research that contained the common theme of epigenetic changes that occurred in the maternal and/or paternal line and that impacted the health of future offspring. This selection of research lends itself to be thought about with respect to public health and raises questions of how this research and information might be used within the field of public health. While not all epigenetic research directly relates to health issues of abnormal health, it is still possible to analyze how this subset of epigenetic research impacts other fields.

The studies I included tested many common concerns that are already deemed a threat to the health status of our population, including issues such as smoking, malnutrition and obesity, alcohol use, and stress. These studies demonstrated that even a short list of factors lead to a vast and complex list of side effects in the health of offspring. These factors are extremely common in our culture, making this research applicable to a large portion of our population. However, it must first be assessed whether or not epigenetics really does

have the potential to be applied to public health, or if this research is not likely to ever extend beyond the field of epigenetics.

A major subdiscipline of public health is epidemiology, which is considered the science of public health. Under the gigantic umbrella of scientific disciplines, many of these disciplines are constantly influencing and advancing because of knowledge gained in another field. This interplay between scientific disciplines is what led me to first analyze how the science of epigenetics might influence the science of epidemiology.

The CDC defines epidemiology as "the study of the distribution and determinants of health-related states or events in specified populations and the application of this study to control health problems" (Thacker 2006). Epidemiology is focused on finding the patterns and spread of disease in a population and epidemiologists track origins, causes, and movement of a disease within a population. Historically, epidemiology was extremely important for understanding the impact and origin of communicable diseases. The goal was to understand the diseases and minimize their impacts on the population as a whole. Some famous examples of epidemiology impacting public health are through its use in the outbreaks of diseases such as polio, small pox, and HIV ("What is Epidemiology?"). However, our modern world is facing a health transition, with the leading causes of death in developed nations being cause by non-communicable and chronic disease. Even more recently, the data show that this epidemiological transformation is not limited to developed nations and even developing nations are seeing the same shift in leading causes of death going from communicable to non-communicable causes ("Health Transition"). This shift is important because this means that the science of epidemiology must change to adapt to this new threat of disease.

This epidemiological transformation is causing health organizations, such as the CDC, to adapt the way they approach major health concerns. Epidemiology is not immune to having to adapt along with the other changes taking place. The CDC states the ways in which they see epidemiology changing to address the new threats to public health as well as how they expect to maintain some of the functions that epidemiology has always provided.

> "CDC epidemiologists will continue to respond to emergent events, be they newly emerging infections, natural disasters, or terrorism, and will continue to study public health problems, such as unintentional injuries, environmental exposures, cardiovascular disease, obesity, tobacco use, and violence domestically and internationally. Public and private partners on the public health team will expand to include new disciplines. The analytic tools and technologies available will increase, and CDC epidemiologists will maintain a critical role in capacity building. Finally, CDC epidemiologists must maintain the scientific integrity the agency has established by remaining rigorous yet adaptable to the challenges new global realities bring to public health (Thacker 2006)."

An important aspect of this quote is that it acknowledges the need to expand on the analytical tools and technologies that epidemiologists use. Epigenetics is an emerging field that, as the research and understanding of the field increases, it can provide helpful insight into the problems public health agencies, such as those the CDC are trying to combat through its incorporation into epidemiology.

As stated in the above quote, CDC epidemiologists are continuing to study public health problems such as cardiovascular disease, obesity, tobacco use, and violence. The public health problems for epidemiologists closely align with the variables that were studied in the epigenetic studies I presented in this paper. Also, all these public health problems are non-communicable health issues, which is consistent with the epidemiological transformation. The prevalence of these public health issues have increased steadily as other communicable disease threats have decreased in prevalence.

To theorize on the possible ways for epidemiology to incorporate and benefit from epigenetics, it is useful to consider how the epigenetics fits in with the epidemiological information for current major population health threats. The obesity epidemic is a major concern for public health and epidemiologists, which is now the leading cause of preventable death in the United States, second only to smoking (Hurt et al. 2011). Epidemiology seeks to understand the total impact of a health problem on a population and epigenetic research has demonstrated impacts of obesity that might not otherwise be linked to obesity. Epigenetics has also helped to confirm some previously observed links. While it is not impossible for epidemiological studies to identify potential cause and effects across generations, these epidemiological studies are limited in the fact that they are all correlational studies. They have the ability to suggest a relationship between two variables, i.e. obesity and health issues in offspring, but they cannot prove that one variable causes a change in the other variable.

Epigenetic research provides epidemiology with a way to further test and strengthen correlational observations. With the issue of obesity, instead of just tracking the disease within a human population and finding trends, epigenetics allows us to perform

experiments that will help further our understanding of what is causing the observations. Epigenetic research has the advantage that it can be performed on organisms besides humans, which helps to alleviate many ethical concerns that epidemiological experiment research would face. For instance, it would be impossible to create an experiment to test the effects of obesity on a population where researchers would induce obesity in a portion of the population and compare it to a control group. However, epigenetic research has the advantage of being able to test on organisms where such a study design is possible and is actually being performed.

Beyond helping to demonstrate the existence of the cause and effect relationships, epigenetic experiments help answer the *why* and the *how* these relationships occur. Epigenetic studies have the ability to demonstrate the changes that occur in the epigenome and how these changes lead to altered gene expression. While not all epidemiological observations are epigenetic in origin, the studies I presented showed that for a single factor like obesity, there could be several different health effects induced by epigenetic mechanisms. For these effects, it is possible for the researchers to track the changes and recognize their effects on an individual. Changes to the epigenome that are observed in model organisms would give researchers enough information to look for similar changes in the epigenomes of humans who have experienced exposures, such as obesity. Applying the observations of the epigenetic changes to the epidemiological observational study will further strengthen the confidence epidemiologists can have in the correlations between two variables because this has added one more dimension of understanding to what might be the actual cause.

Epigenetic research is important in not only helping to explain correlation, but also because it can provide insight into factors that might be influencing an epidemic. For any health issue, there are factors that influence the health of the population directly by members of the population experiencing these factors during their lifetime. With obesity, we understand that there are things about our current culture and different subpopulations that influence the prevalence of the condition. Obvious disease determinants of obesity are lack of activity levels, the types of food we eat, and the amount of food we eat. Observations like this, and the study of the factors that are currently affecting the population, fall within the realm of epidemiology.

However, epigenetic research reveals that there might be other determinants of disease that influence the prevalence of a condition within a particular population. Epigenetic research, in combination with epidemiological research, can work to create a better understanding on how our vulnerability to a certain condition can increase in a population over the course of generations. Epigenetic research has demonstrated that obesity in parents can lead to changes in the offspring's epigenome. Therefore, epigenetic research is needed to compliment what epidemiologists already know and to fully understand what these changes mean to these offspring and the population.

In cases like the obesity epidemic, where the prevalence continues to increase in our population, more and more offspring will be born to obese parents and so a greater proportion of the population will be born with changes to their epigenome brought on by parental obesity. Epigenetic research shows that because of these changes, the offspring are at a greater predisposition for obesity. With this information, epidemiologists can recognize that with each passing generation, our population as a whole may be more

vulnerable to the more obvious disease determinants that are influencing the increase of obesity rates that I mentioned previously. This type of interplay between traditional epidemiology and epigenetics is not exclusive to obesity. Incorporating epigenetic factors as a determinant of disease will only help epidemiologists gain a more complete idea of the spread of a health condition.

Epigenetic research demonstrates a variety of ways that events that happened to our parents can increase our susceptibility to certain conditions. I just discussed how a parent experiencing obesity in their lifetime could translate to epigenetic effects in offspring that make them more likely to experience obesity in their lifetime, which is an event in a parent's life leading to a similar experience in the child's life. However, as some of the studies I've included demonstrate, the events that our parents experience which induce epigenetic changes do not always lead to the changes in the offspring that directly reflect the experiences of the parents. Parents can sometimes have experiences in their lifetimes that lead to an increase risk in offspring for a seemingly unrelated condition in their offspring. An example of this was the paternal nutritional studies, where varied food intake in paternal grandparents and fathers had the ability to change the incidence rates of diabetes and cardiovascular disease in offspring.

Knowing the variety of possible epigenetic effects that are caused by different health problems will help epidemiologists to achieve their goal of understanding the true breadth to which a health issue affects a population. In the studies on paternal nutrition leading to increases in diabetes and cardiovascular disease, the research demonstrates increased risks to the health status of future generations that might otherwise not be noticed. In addition to knowing that future generations of the obesity epidemic might be at a greater

predisposition, these studies demonstrate that these same individuals are at a higher risk for diabetes and cardiovascular disease.

This type of research can also be used to understand patterns in a current population as well. For instance, if in the current population there is a huge rise in the rates in diabetes and cardiovascular disease, epigenetic research of this type allows epidemiologists to look beyond just current influences on an individual's health. Using medical histories, epidemiologists already have the ability to recognize correlations between events in an individual's family history and the increase of disease in an offspring. However, epigenetic research allows for the recognition of specific changes in the genome that are present if your parent's medical histories are impacting your current health. Using that information, epidemiologists could compare if major increases in rates of a health condition correspond to a family induced vulnerability or if current, environmental factors are more to blame.

Research on parental epigenetic effects has demonstrated that the effects of environmental factor are not always clear-cut. If the effects are in any way sex-linked or sex-specific, the effects between genders and how they are transmitted can vary greatly in type and severity. Epidemiologists need to recognize that there are effects that are heritable through either maternal or paternal lines only, information that is provided through epigenetic research. With this information, they would know which line could be more affected by a certain environmental factor and what side might need more attention placed on it. If a certain environment factor leads to health issues that are heritable through the paternal line only, epidemiologists would know to track the issues through the paternal line and save resources by not spending them on the maternal line. Epidemiologists could

focus preventative measures on whatever gender is more susceptible to damage by these exposures to minimize the effects to future generations.

When environmental factors are creating health problems, understanding the epigenetic mechanisms of transmission will allow us to understand why there are varying levels of impacts between sexes. Even if effects are not heritable through one gender-line, some effects will be more pronounced one gender than the other regardless of which parent the effects were inherited through. If only one gender of future generations is experiencing health problems, it might be easy for epidemiologists to not immediately recognize the impact of previous exposures. Knowing how epigenetic effects appear in different genders over the course of generations would allow epigenetics to pay attention to the differences in health that might arise between the genders. The epigenetics will add to epidemiology in helping to explain why these differences arise as well. Not only for effects that appear in only one gender, but also to how they are transmitted through a single gender's germline in some instances.

One of the continual goals of epidemiology is to provide data for directing public health action, such as the creation of public health policy ("What is Epidemiology?"). If epigenetics has an impact on and is incorporated into epidemiological work, the connection between epidemiology and public health provides a bridge for epigenetics to also impact public health policy.

Public health policy works to create initiatives that can make a large impact on the heath status of a community. To create such policies, there needs to be a large body of research proving that there is a significant need for these policies to be implemented. However, having research and data isn't always enough to guarantee the creation of

policies. Even when there is research to prove that these policies could create a significant impact, this research doesn't always lead to the creation of policies that are enacted and enforced (Brownson et al. 2009). Typically, when a public health policy is being created, the policy will have a sponsor to push the policy through and these sponsors are the ones who determine what information is used to support the policy. For the public health policies that are enacted, the policies do not always cite scientific research as the need for their implementation or the creation of their guidelines. In fact, one source on the creation of evidence-based public health policy states that only 6.5% of sponsors for today's major public health policies provided details that the law was based on scientific research to (Brownson et al. 2009). Even with such limited amount of directly cited scientific research, the authors suggest that scientific research has the power to influence public health policies through more indirect routes. These indirect routes would involve the research influencing policies in a non-linear model; instead of research leading to direct policy change, the research would serve to create gradual enlightenment on the subject at hand. Epigenetic research has the potential to influence public health policy through both directly cited research and more indirect routes.

Many of the policies for public health that are already in place came about without epigenetic research because there is a large enough body of knowledge from other fields to recognize their significance. For example, the Surgeon General has issued an advisory on alcohol use in pregnancy. In this warning, the U.S. Surgeon General cites many negative effects of alcohol use in pregnancy to fetal defects (Carmona 2005). While not as extreme of a stance, the CDC has also issued a statement on the risks of smoking while pregnant ("Tobacco Use and Reproductive Outcomes"). Without epigenetic research, there is still

plenty of support to create an official stance against maternal drinking and smoking. However, there is a lack of an official stance on whether or not men should smoke or drink while trying to conceive with their partner.

Several of the studies I presented in this paper have made a compelling case that there might be a reason for such an official stance to be created because these activities by a father can cause detrimental health effects in their offspring. Unlike maternal drinking and smoking, paternal effects in these categories are not as obvious. Epigenetic research is the most powerful evidence we have to demonstrate the negative effects the father can have on offspring if they drink and smoke. Without the epigenetic research that demonstrates the ability for sperm to transmit changes in the epigenome, conceptualizing means of paternal influence on an offspring health is extremely difficult. The idea that men have a responsibility to change their behaviors when trying to conceive a child in not commonplace in our culture, arguably because there has never been a reason to think the father can have much influence on the offspring's health if it isn't strictly a genetic issue.

To create a public health policy for paternal smoking and drinking, sponsors of such policies would to rely heavily on the epigenetic research, and this research would directly influence policy creation. The research would have to demonstrate both the means of impact by the father and that this impact is significant enough to a threat to public health status. Such a policy could not be created without this body of research, because unlike the effects of maternal smoking and drinking, the sponsors to create a policy against these paternal behaviors would have no evidence from other disciplines to demonstrate the negative health outcomes in offspring.

While there are existing policies on maternal smoking and drinking during pregnancy, this does not mean that epigenetic research has no role in these public health policies. In cases where there are other disciplines that demonstrate a relationship between two variables, epigenetic research would help supplement the existing research rather than heading the creation of public health policy. The addition of the epigenetic research would add another dimension to the understanding of the impact these behaviors have on offspring health. This extra evidence could help maintain already implemented public health policies. An important aspect of public health policies is the surveillance and evaluation of implemented programs and policies. The health of our population is constantly changing, so it is important to make sure there is enough evidence to warrant the continuation of policies. A key element in public health policy surveillance is to continue to analyze current data to confirm that research on the topic is continuing to demonstrate the importance of the policy (Allaki et al. 2013). The addition of epigenetic research to the existing research that was used in policy creation would create a more complete picture of the total impact on public health status. The more that is known about the true threat to public health status, the more likely a policy is to be maintained.

While epigenetics can play a role in some aspects of public health, some epigenetic effects demonstrated in these studies are caused by factors too specific to create any new impact on public health. Part of creating public health policy is to create policies that provide the largest impact for the improvement of public health with the money and resources spent (Brownson et al. 2009). Some of the effects demonstrated in the research are limited to a small portion of the population and are not distinct enough to warrant policies beyond what are already in place. One specific case was that variations in

nutritional intake during the male's slow growth period (SGP) lead to different health issues in both their sons and grandsons. While these studies demonstrate compelling evidence of the vulnerability of males during their slow growth period and the effects this can have on future generations, it is unlikely this information would lead to new policies. Already on the public health agenda are policies and programs to reduce childhood obesity rates ("Childhood Overweight and Obesity") and decrease childhood hunger ("United Nations Millennium Development Goals"). These existing policies would already include males during their slow growth periods as well as all other children. Having these policies could help minimize the effects demonstrated in the research of males during their SGP, even though the policy is not specifically designed based on the epigenetic research. For this reason, creating a policy that focused just on males during their slow growth period in addition to already existing policies seems incredibly unlikely because it would not create the largest improvement with the resources spent.

The integration of some aspects of parental epigenetic effects into public health certainly seems possible, but there are some obstacles to overcome before this can occur. A challenge that plagues the field of public health is how difficult it can be for proven, scientific research to be implemented in a real-world setting (Peters et al. 2013). Assuming that the avenues I have presented in this paper are feasible ways for epigenetic research to be used in epidemiology and public health, it could still take years before any of the research makes its way into public health research and policies. Hopefully, as evidence demonstrating the influence parental epigenetic effects have on public health status continues to get stronger, the research's importance will be fully appreciated.

The field of epigenetics has advanced considerably since Conrad Waddington first proposed the term in the mid-20th century. From Waddington's initial concept of epigenetics grew a variety of research, including a broad body of research demonstrating the existence of parental epigenetic effects. While our understanding of parental epigenetic effects is still expanding, we know that the experiences parents have in their lifetime can play a huge role on an offspring's health. Some of the effects demonstrated in the research certainly have the power to negatively impact population health status and become a concern for public health. While it is impossible to know ultimately how parental epigenetics and public health will interact, it is almost certain that the two disciplines will cross paths.

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